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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
09/997,504	11/19/2001	Michael E. Himmel	NREL 99-38	3921

23712 7590 06/29/2005

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EXAMINER	
RAO, MANJUNATH N	

ART UNIT	PAPER NUMBER
1652	

DATE MAILED: 06/29/2005

Please find below and/or attached an Office communication concerning this application or proceeding.

Office Action Summary	Application No.	Applicant(s)	
	09/997,504	HIMMEL ET AL.	
	Examiner	Art Unit	
	Manjunath N. Rao, Ph.D.	1652	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTH(S) FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If the period for reply specified above is less than thirty (30) days, a reply within the statutory minimum of thirty (30) days will be considered timely.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, causa tha application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing data of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☒ Responsive to communication(s) filed on *18 April 2005*.

2a)☐ This action is **FINAL**. 2b)☒ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4)☒ Claim(s) *3,7,29 and 31* is/are pending in the application.

4a) Of the above claim(s) _____ is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) *3,7,29 and 31* is/are rejected.

7)☐ Claim(s) _____ is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

9)☒ The specification is objected to by the Examiner.

10)☒ The drawing(s) filed on *19 November 2001* is/are: a)☒ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

11)☐ The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

Priority under 35 U.S.C. § 119

12)☒ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☐ All b)☐ Some * c)☒ None of:

1.☒ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

1) ☒ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☐ Information Disclosure Statement(s) (PTO-1449 or PTO/SB/08) Paper No(s)/Mail Date _____.
4) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date. _____ .
5) ☐ Notice of Informal Patent Application (PTO-152)
6) ☐ Other: _____.

S.O.D

DETAILED ACTION

CONTINUED EXAMINATION UNDER 37 CFR 1.114 AFTER FINAL REJECTION

A request for continued examination under 37 CFR 1.114, including the fee set forth in 37 CFR 1.17(e), was filed in this application after final rejection. Since this application is eligible for continued examination under 37 CFR 1.114, and the fee set forth in 37 CFR 1.17(e) has been timely paid, the finality of the previous Office action has been withdrawn pursuant to 37 CFR 1.114. Applicant's submission filed on 4-18-05 has been entered.

Claims 3, 7, 29, 31 are still at issue and are present for examination.

Applicants' amendments and arguments filed on 4-18-05 have been fully considered and are deemed to be persuasive to overcome the rejections previously applied. Rejections and/or objections not reiterated from previous office actions are hereby withdrawn.

Priority

Applicant's claim for domestic priority under 35 U.S.C. 119(e) is acknowledged. Acknowledgment is made of applicant's claim for foreign priority based on an application filed in WIPO on 5-19-2000. It is noted, however, that applicant has not filed a certified copy of the PCT/US00/13971 application as required by 35 U.S.C. 119(b).

Sequence Compliance

Applicant is required to comply with the sequence rules by inserting the correct sequence identification numbers of all sequences recited within the claims and/or specification. It is

particularly noted that the sequence identification numbers recited in claim 7 and 31 are listed as short oligonucleotide sequences as opposed to a polypeptide sequences as indicated in the claims. See particularly 37 CFR 1.821(d). Because of improper sequence identification numbers, Examiner was unable to do a meaningful search.

Claim Rejections - 35 USC § 112

The following is a quotation of the second paragraph of 35 U.S.C. 112:

> The specification shall conclude with one or more claims particularly pointing out and distinctly claiming the subject matter which the applicant regards as his invention.

Claim 7 which depends from claim 3 is rejected under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention. Claim 7 recites the method of claim 3 wherein the mutant glycosyl hydrolase comprises SEQ ID NO:2-Y245G, SEQ ID NO:3 Y42R, SEQ ID NO:4 W82R. This is confusing because it is not clear as to what the specific amino acids next to the SEQ IDs represent. Furthermore it cannot be concluded that the amino acid positions recited refer to the glycosyl-stabilizing amino acid recited in claim 3, because claim 3 states that the glycosyl-stabilizing amino acid comprises tyrosine (Y) and the replacing amino acid comprises glycine (G). The amino acids referred to in SEQ ID NO:3 and 4 are not glycine and tyrosine. Examiner requests clarification.

Claims 7 and 31 are rejected under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention. Claim 7 and 31 recite that the glycosyl hydrolase comprises SEQ ID

NO:2-Y245G, SEQ ID NO:3 Y42R, SEQ ID NO:4 W82R or SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4. However, a perusal of the sequence listing indicates that these sequences are actually short oligonucleotide sequences. Therefore it is not clear as to which specific sequence applicants are referring to and because of this discrepancy, Examiner was unable to do a meaningful search.

Claims 29 and 31 are rejected under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention. Claims 29 and 31 are drawn to a method of increasing the specific activity of *A.cellulolyticus* E1 endoglucanase by replacing through site-directed mutagenesis an active site associated glycosyl-stabilizing tyrosine amino acid with glycine. It is not clear to the Examiner as to how those skilled in the art would know as to which specific amino acid in the full length sequence of the above enzyme should be targeted for substitution. Without the specific amino acid known, it would be meaningless for those skilled in the art to even attempt this method. Examiner requests clarification.

The following is a quotation of the first paragraph of 35 U.S.C. 112:

> The specification shall contain a written description of the invention, and of the manner and process of making and using it, in such full, clear, concise, and exact terms as to enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the same and shall set forth the best mode contemplated by the inventor of carrying out his invention.

Claims 3, 29, 31 are rejected under 35 U.S.C. 112, first paragraph, because the specification, while being enabling for a method of increasing the specific activity of EI endoglucanase comprising the amino acid sequence of SEQ ID NO: 10, 12, 14 (SEQ ID NO:2, 3, 4?) isolated

from *Acidothermus cellulolyticus* on pretreated biomass, comprising the replacement of the specific active site associated glycosyl stabilizing amino acid, i.e., Y at position 245 with G in SEQ ID NO:10, or Y at position 42 with R in SEQ ID NO:12, or W at position 82 with R in SEQ ID NO:14 (SEQ ID NO:2, 3, 4 ?) does not reasonably provide enablement for a method of increasing the specific activity of any glycosyl hydrolase enzyme isolated from any or all sources acting on any or all type of substrates, comprising the replacement of any tyrosine that is "active site associated" and "glycosyl stabilizing" with a glycine amino acid. The specification also does not reasonably provide enablement for a method of increasing the specific activity of any glycosyl hydrolase enzyme isolated from any or all strains of *A.cellulolyticus* by site-directed mutagenesis such that an active site associated glycosyl-stabilizing amino acid of the endoglucanase is replaced with an amino acid, the replacing amino acid binding cellobiose less tightly than the glycosyl-stabilizing amino acid, to provide a mutant endoglucanase wherein said glycosyl-stabilizing amino acid comprises a tyrosine and the replacing amino acid comprises a glycine. The specification does not enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make the invention commensurate in scope with these claims.

Factors to be considered in determining whether undue experimentation is required, are summarized in *In re Wands* (858 F.2d 731, 8 USPQ 2nd 1400 (Fed. Cir. 1988)) as follows: (1) the quantity of experimentation necessary, (2) the amount of direction or guidance presented, (3) the presence or absence of working examples, (4) the nature of the invention, (5) the state of the prior art, (6) the relative skill of those in the art, (7) the predictability or unpredictability of the art, and (8) the breadth of the claim(s).

Claims 3, 29, 31 are so broad as to encompass a method of increasing the specific activity of any glycosyl hydrolase enzyme isolated from any or all sources acting on any or all type of substrates, comprising the replacement of any active site associated glycosyl stabilizing amino acid and a method of increasing the specific activity of any glycosyl hydrolase enzyme isolated from any or all strains of *A.cellulolyticus* by site-directed mutagenesis such that an active site associated glycosyl-stabilizing amino acid of the endoglucanase is replaced with an amino acid, the replacing amino acid binding cellobiose less tightly than the glycosyl-stabilizing amino acid, to provide a mutant endoglucanase wherein said glycosyl-stabilizing amino acid comprises a tyrosine and the replacing amino acid comprises a glycine. The scope of the claims is not commensurate with the enablement provided by the disclosure with regard to the application of a single method for an extremely large number of glycosyl hydrolases broadly encompassed by the claims and with regard to the analyzing and testing of the extremely large number of mutants that result from the site-directed mutagenesis experiment on *A.cellulolyticus* endoglucanase. Since the amino acid sequence of a protein determines its structural and functional properties, predictability of which changes can be tolerated in a protein's amino acid sequence and obtain the desired activity requires a knowledge of and guidance with regard to which amino acids in the protein's sequence, if any, are tolerant of modification and which are conserved (i.e. expectedly intolerant to modification), and detailed knowledge of the ways in which the proteins' structure relates to its function. However, in this case the disclosure is limited to the single method of making a variant by changing the amino acid Y at position 245 to G in SEQ ID NO:10, or Y at position 42 to R in SEQ ID NO:12, or W at position 82 to R in SEQ ID NO:14 (SEQ ID NO:2, 3, 4 ?). It would require undue experimentation of the skilled

artisan to make and use the claimed method to increase the specific activity of any or all glycosyl hydrolases including structural analogs of endoglucanases. The specification is also limited to the single method of enhancing the specific activity of a single EI endoglucanase against a single substrate, i.e., pretreated biomass, but provides no guidance with regard to using the same method on any glycosylhydrolase isolated from any source and having any structure or with regard to the analysis of the extremely large number of mutants and variants that arise from the site-directed mutation of *A.cellulolyticus* endoglucanase. In view of the great breadth of the claim, amount of experimentation required to use the above method on any glycosyl hydrolase which includes a large number of different hydrolytic enzymes acting on a wide range of substrates, the lack of guidance, working examples, and unpredictability of the art in predicting function from a polypeptide primary structure (e.g., see Ngo et al. in The Protein Folding Problem and Tertiary Structure Prediction, 1994, Merz et al. (ed.), Birkhauser, Boston, MA, pp. 433 and 492-495, Ref: U, Form-892), the claimed invention would require undue experimentation. As such, the specification fails to teach one of ordinary skill how to use the full scope of the method encompassed by these claims.

While recombinant and mutagenesis techniques are known, and it is routine in the art to screen for multiple substitutions or multiple modifications, as encompassed by the instant claims, the positions within a protein's sequence where amino acid modifications can be made with a reasonable expectation of success in obtaining the desired activity/utility are limited in any protein and the result of such modifications is unpredictable. In addition, one skilled in the art would expect any tolerance to modification for a given protein to diminish with each further and additional modification, e.g. multiple substitutions.

The specification does not support the broad scope of the claims which encompasses a method of increasing the specific activity of any glycosyl hydrolase enzyme isolated from any or all sources acting on any or all type of substrates, comprising the replacement of any active site associated glycosyl stabilizing amino acid because the specification does not establish that: (A)the method works in all or any glycosyl hydrolases which includes a large number of different enzymes; (B) the specific activity of the glycosyl hydrolases is enhanced irrespective of the type of substrate; (C) a rational and predictable scheme for identifying and modifying any active site residue in any glycosyl hydrolase with an expectation of obtaining the desired biological function; and (D) the specification provides insufficient guidance as to which of the essentially infinite possible choices is likely to be successful.

Thus, applicants have not provided sufficient guidance to enable one of ordinary skill in the art to use the claimed invention in a manner reasonably correlated with the scope of the claims broadly including all or any glycosyl hydrolase and all or any type of substrates. The scope of the claims must bear a reasonable correlation with the scope of enablement (In re Fisher, 166 USPQ 19 24 (CCPA 1970)). Without sufficient guidance, determination of a method that applies to all types of glycosyl hydrolases and all types of substrates is unpredictable and the experimentation left to those skilled in the art is unnecessarily, and improperly, extensive and undue. See *In re Wands* 858 F.2d 731, 8 USPQ2nd 1400 (Fed. Cir, 1988).

In response to the previous Office action, applicant has traversed the above rejection arguing that, "...The Office notes that Applicants have shown an improvement in activity by replacing tyrosine at position 245 in glycohydrolase from *A. cellulolyticus*. but have not provided knowledge or guidance as to how this might be done outside the limited scope

of the enabled species" and that "Such knowledge may be found by way of examples in paragraph (0025) of the published Application and in the table there following, which shows analogous substitution sites in glycosyl hydrolase family enzymes". While Examiner agrees that applicant has support for such specific examples and is enabled for those specific analogous, he respectfully disagrees that such guidance is enough for claiming a method of increasing the specific activity of the entire group of glycosylhydrolases. It must be remembered that the family of glycosylhydrolases includes a variety of hydrolases which have separate class of substrates and results in generation of a variety of products. The examples provided by the applicant is specific for endoglucanases only but not all members of glycosyl family.
Applicant also argues that "the referenced page provides, by way of examples a rationale or theory for making such substitutions in a genus as have been shown to work in the illustrated species and that additional proof that applicant s were in possession of the theory leading to effective modifications of the enzyme at the time of filing may be found in kinetics discussion of paragraph 0058. Again while applicants may have provided a theory or a rationale for making amino acid changes they have not unequivocally shown that said rationale would be effective on every member of glycosylhydrolase family. As rightly pointed out by the applicant, the determination of what constitutes undue experimentation in a given case requires the application of a standard of reasonableness, having due regard for the nature of invention and the state of state of art" and that is what examiner has exactly done here. It can be concluded, at the most, applicant has provided support for a method of increasing the specific activity of a specific endoglucanase but not a method for increasing the specific activity of all members of the complex group of enzymes included in the large family, "glycosyl hydrolases". As stated

earlier, applicant's arguments are not persuasive because while methods to produce variants of a known sequence such as site-specific mutagenesis, random mutagenesis, etc. are well known to the skilled artisan producing method of increasing the specific activity of all members of glycosyl hydrolases as claimed by applicants requires that one of ordinary skill in the art know or be provided with a universal method for the selection of specific amino acid residues in each and every member of the glycosyl hydrolase family and/or provide a single universal method to identity those specific mutants and variants that arise from site-directed mutagenesis experiments to be comprised with a substituted glycosyl-stabilizing amino acid as claimed in claim 29. Without such guidance one of ordinary skill would be reduced to the necessity of producing and testing all of the virtually infinite possibilities. This would clearly constitute undue experimentation. While enablement is not precluded by the necessity for routine screening, if a large amount of screening is required, the specification must provide a reasonable amount of guidance with respect to the direction in which the experimentation should proceed. Such guidance has not been provided in the instant specification. Hence the above rejection is maintained.

Claim 3 is rejected under 35 U.S.C. 112, first paragraph, as containing subject matter which was not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventor(s), at the time the application was filed, had possession of the claimed invention.

Claim 3 is directed to a method of enhancing the specific activity of any glycosyl hydrolase. Claim 3 is rejected under this section of 35 USC 112 because the claim is directed to

a method of using a genus of polypeptides that have not been disclosed in the specification. No description has been provided of the polypeptide sequences encompassed by the claim. No information, beyond the characterization of a single specific polypeptide having EI-endoglucanase activity and isolated from *A.cellulolyticus* (in Example 7) has been provided by applicants which would indicate that they had possession of the claimed genus of polypeptides. The specification does not contain any disclosure of the structure of the polypeptide sequences within the scope of the genus to be used in the claimed method. The genus of polypeptides is a large variable genus including peptides which can have a wide variety of structures. Therefore many structurally unrelated polypeptides are encompassed within the scope of these claims. The specification discloses a method for only a single species of the genus which is insufficient to put one of skill in the art in possession of the attributes and features of all species within the genus for use in the claimed method. Therefore, one skilled in the art cannot reasonably conclude that applicant had possession of the claimed invention at the time the instant application was filed.

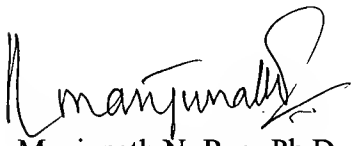
Applicant is referred to the revised guidelines concerning compliance with the written description requirement of U.S.C. 112, first paragraph, published in the Official Gazette and also available at www.uspto.gov.

Conclusion

None of the claims are allowable.

Any inquiry concerning this communication or earlier communications from the Examiner should be directed to Manjunath N. Rao, Ph.D. whose telephone number is 571-272-0939. The Examiner can normally be reached on 7.00 a.m. to 3.30 p.m. If attempts to reach the

examiner by telephone are unsuccessful, the Examiner's supervisor, Ponnathapura Achutamurthy can be reached on 571-272-0928. The fax phone numbers for the organization where this application or proceeding is assigned is 571-273-8300 for regular communications and for After Final communications. Any inquiry of a general nature or relating to the status of this application or proceeding should be directed to the receptionist whose telephone number is 571-272-1600.



Manjunath N. Rao, Ph.D.
Primary Examiner
Art Unit 1652

June 23, 2005